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## Visualization and Modelling of the Thermal Inactivation of Bacteria in a Model Food

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**A large number of incidents of food poisoning have been linked to undercooked meat products. The use of mathematical modelling to describe heat transfer within foods, combined with data describing bacterial thermal inactivation, may prove useful in developing safer food products while minimizing thermal overprocessing. To examine this approach, cylindrical agar blocks containing immobilized bacteria (*Salmonella typhimurium* and *Brochothrix thermosphacta*) were used as a model system in this study. The agar cylinders were subjected to external conduction heating by immersion in a water bath. They were then incubated, sliced open, and examined by image analysis techniques for regions of no bacterial growth. A finite-difference scheme was used to model thermal conduction and the consequent bacterial inactivation. Bacterial inactivation rates were modelled with values for the time required to reduce bacterial number by 90% (D) and the temperature increase required to reduce D by 90% taken from the literature. Model simulation results agreed well with experimental results for both bacteria, demonstrating the utility of the technique.**

Food-borne diseases continue to be a problem of global concern. Active surveillance of infections caused by bacterial food-borne pathogens in the United States revealed 130,000 culture-confirmed cases in the population in 1997 (3). Confirmed cases represent only a fraction of the total, and it was estimated that the true incidence was likely to have been approximately 8 million cases. In the United Kingdom, the incidence of food-borne illness continues to increase, and in 1997 over 94,000 cases were reported (8). Poultry, eggs, and red meat products are frequently identified as vehicles of food poisoning, and the most common factors contributing to outbreaks include inappropriate storage of food, cross-contamination, and inadequate cooking or reheating (21).

Consumer demand for fresher, more natural food has resulted in a trend towards milder methods of food processing that inactivate microbes without having a deleterious effect on food quality. In this context, an ability to predict the safety margins of inactivation or preservation processes becomes particularly important. Mathematical modelling has been used to assist food process engineers in optimizing sterilization or pasteurization processes. In particular, much effort has focused on modelling the sterilization of canned foods (14, 26, 27). Teixeira et al. (26) presented a numeric technique for computationally determining spore survival distribution spatially within a can exposed to conduction heating. Banga et al. (4) used finite-difference and finite-element methods to model the conduction heating of canned tuna and demonstrated good agreement between theoretical and experimental temperature profiles. As a consequence of the presence of *Escherichia coli* O157:H7 in undercooked hamburger patties, Vijayan et al. (29) modelled the inactivation of this bacterium in frozen patties during frying using a finite-difference scheme. It was those researchers' opinion that such computational modelling may be useful in developing safe cooking processes.

Mathematically, the inactivation of bacteria has traditionally been expressed with the well-known concepts of D and z values (the time [in seconds] required to reduce bacterial number by 90% and the increase in temperature [in kelvins] required to reduce D by 90%, respectively) (13). These values are available in the literature for all commonly occurring food poisoning and food spoilage bacteria in a range of foods. Much work has been done to examine the heat resistance of pathogenic bacteria such as *E. coli* O157:H7 (2, 15), *Salmonella* spp. (25), and *Listeria monocytogenes* (11, 25). The majority of such studies have been conducted with homogenized foods or liquid media, without any consideration of the spatial (three-dimensional) element of solid foods as they are heated.

In spite of all the research conducted on modelling heat conduction in packaged foods and the corresponding experimental work performed with pathogenic bacteria, relatively very little research combining the two has been performed. Some work, however, has been performed with bacterial spores or enzymes as time-temperature indicators. Brown et al. (7) prepared alginate and pureed food cubes inoculated with bacterial spores. Mathematical modelling was used to predict heat transfer and the consequent spore destruction as particles were heated. Significant deviations were found between experimental and theoretical results. Teixeira et al. (28) used a model to simulate heat conduction through cans of pea puree inoculated with *Bacillus stearothermophilus* spores. Experimental results attained for spore destruction were used to determine the heat resistance of these spores. Results compared well with those in the literature from isothermal spore inactivation experiments with pea puree (24). Bhamidipati and Singh (6) and Ramaswamy et al. (23) used enzymes (horseradish peroxidase and bovine pancreas trypsin, respectively) embedded in particles heat treated in a liquid flow system to validate the process of numeric modelling. Both studies reported good agreement between values for predicted and measured retention of enzyme activity.

Conventional thermal-processing calculations assume that the heat resistance of microbes under changing conditions can be predicted from their behavior at static temperatures. This

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assumption has been shown not to be true for a number of vegetative bacteria, including *Salmonella typhimurium* (17) and *L. monocytogenes* (22), whose resistance can increase during heating at slowly rising temperatures. It is our opinion that the heating rates typically observed during the conduction heating of foods are too fast to allow vegetative microorganisms sufficient time to acquire any increased heat resistance. This study will, in part, test this hypothesis for a food simulant exposed to external conduction heating.

The aim of this collaborative work between researchers in Reading and Birmingham, United Kingdom, was to establish how predictive microbiology can be applied in engineering environments. The thermal inactivation of the food-borne pathogen *S. typhimurium* and meat spoilage organism *Brochothrix thermosphacta* immobilized in agar cylinders simulating sausages subjected to external conduction heating was studied (1). Cylinder slices were examined by image analysis techniques for regions of bacterial inactivation, and results were compared to those from mathematical modelling. Numeric simulations were also performed to examine inactivation profiles of *E. coli* O157:H7 in lean ground beef sausages.

#### MATERIALS AND METHODS

**Organisms and growth conditions.** *S. typhimurium* LT2 (NCIMB 10248) and *B. thermosphacta* MR165 (NCIMB 702891) were maintained on glass beads at  $-70^{\circ}\text{C}$ . Cultures of *S. typhimurium* for thermal inactivation experiments were produced as follows: 10 ml of tryptone soya broth (Oxoid, Basingstoke, United Kingdom) was inoculated with a single colony taken from a tryptone soya agar (TSA) plate and incubated for 7 to 8 h at  $37^{\circ}\text{C}$ . The culture was then diluted 1:500 into fresh broth and incubated on a shaking platform for a further 22 to 24 h. *B. thermosphacta* cultures were obtained by inoculating 100 ml of brain heart infusion (BHI) broth (Difco Laboratories) with a colony taken from BHI agar and incubating the inoculated broth at  $25^{\circ}\text{C}$  for 16 h.

**Preparation and inoculation of agar cylinders.** Dialysis tubing (27-mm diameter; Sigma Chemical Co.) was rinsed in running water to remove glycerol and cut into 0.35-m lengths. The tubing was knotted at one end and sterilized by autoclaving. Flasks containing 150 ml of molten TSA (for *S. typhimurium*) at  $50^{\circ}\text{C}$  or BHI agar (for *B. thermosphacta*) at  $45^{\circ}\text{C}$  were inoculated with 1.5 ml of culture. After being mixed, the agar was quickly poured into the sterile dialysis tubing through a funnel and the open end was sealed by tying it with string. The tubing was hung vertically at room temperature (approximately  $25^{\circ}\text{C}$ ) to allow the agar to set. The agar cylinders so produced were of uniform diameters.

**Thermal inactivation experiments.** Thermal inactivation experiments were performed with the agar cylinders encased within dialysis tubing. Cylinders inoculated with *S. typhimurium* were heated not long after the agar had set (i.e., within approximately 1 h). The agar cylinders were heated for different times by submerging them in a circulating water bath set at  $70^{\circ}\text{C}$ . After heat treatment, the cylinders were cut into lengths of 2 to 3 cm and the sections were incubated overnight (for 18 to 22 h) at  $37^{\circ}\text{C}$ . The protocol for *B. thermosphacta* was similar except that the agar cylinder was held at  $5^{\circ}\text{C}$  for 1 h before being heated and the heating temperature used was  $60^{\circ}\text{C}$ . All thermal inactivation experiments were conducted in duplicate.

**Image analysis of cylindrical agar slices.** After incubation, the agar blocks were cut into smaller slices with thicknesses of 5 to 6 mm and analyzed for regions of bacterial growth or destruction by an image analysis system (Photonics Science Ltd., E. Sussex, United Kingdom). Cylinder slices were placed on a lighting stage in a dark box. Pictures were taken of these slices with a charge-coupled-device camera. A dark circular region of bacterial growth and a clearer zone around it where the bacteria had been inactivated could be observed towards the center of the slice (Fig. 1). Image-Pro Plus Imaging software (Media Cybernetics, Silver Spring, Md.) was used to analyze these pictures. The dark circular region was manually traced out with the computer mouse, and the radius of the growth zone ( $R_{\text{growth}}$ ) (in meters) as a fraction of the slice radius ( $R$ ) was evaluated by the imaging software. The ratio evaluated by the use of this technique was found to be highly reproducible, as when the same slice was examined by this technique, differences in readings were found to be less than 1%. Each slice was analyzed three times, and means were taken.

**Mathematical modelling.** Heat conduction within a cylinder can be described by the cylindrical form of the heat conduction equation (9):

$$\frac{\partial T}{\partial t} = \alpha \left[ \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial T}{\partial r} \right) + \frac{\partial^2 T}{\partial z^2} \right] \quad (1)$$

where  $\alpha$  is the thermal diffusivity,  $T$  is the temperature (in kelvins),  $t$  is time (in seconds), and  $r$  is the radial coordinate (in meters). As the agar sausages were approximately 95% water by weight, a value for  $\alpha$  that was the same as that of

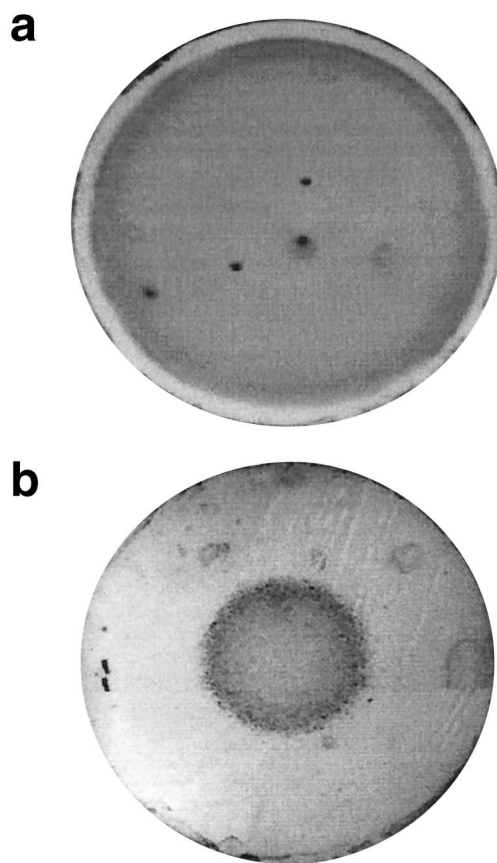


FIG. 1. Pictures show slices taken from agar cylinders which had been inoculated with *S. typhimurium* and then heated. Darker regions indicate zones where bacteria have grown. (a) Slice from a cylinder heated for 90 s; (b) slice from a cylinder heated for 360 s.

water ( $1.45 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ ) was used. Numeric simulations were also performed for heat conduction in a ground beef sausage, for which a value for  $\alpha$  of  $1.26 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$  was used (10). It was also assumed that the dialysis tubing had little influence on heat transfer into the agar, as the tubing was both relatively thin (less than 100  $\mu\text{m}$ ) and water permeable. Equation 1 was solved by using a finite-difference scheme with the boundary conditions described below. For cylinders undergoing conduction heating, the following boundary conditions are commonly used:

$$\text{at } r = 0, \partial T / \partial r = 0 \quad (2)$$

and

$$\text{at } r = R, z = 0 \text{ or } L, q = h(T_{\text{liquid}} - T_{\text{surface}}) \quad (3)$$

where equation 2 describes the symmetry of the system and equation 3 describes the interfacial heat transfer between surface and surroundings. In these equations,  $L$  is the sausage length (in meters),  $q$  is the heat flux into the sausage (in watts per square meter),  $h$  is the local heat transfer coefficient (in watts per square meter per kelvin), and  $T_{\text{liquid}}$  and  $T_{\text{surface}}$  are the temperatures (in kelvins) of the liquid and surface, respectively. In experiments with equation 3, heating is provided by water undergoing forced circulation. The heating rate is thus controlled by internal conduction and does not depend on the heat transfer coefficient (i.e., the value of  $h$  is high). Therefore, instead of the boundary condition written for equation 3, the following approximation was used:

$$T_{\text{surface}} = T_{\text{liquid}} \quad (4)$$

In conjunction with the modelling of heat conduction, bacterial destruction throughout the cylinder was modelled as the temperature dynamically varied, by the following equation (29):

$$\frac{dN}{dt} = \frac{2.303}{D_{\text{ref}}} 10^{(T - T_{\text{ref}})/z} N \quad (5)$$

TABLE 1. D and z values of bacteria considered in this study

Bacterium	Reference	$T_{\text{ref}}$ (°C)	$D_{\text{ref}}$ (s)	$z$ (°C)
<i>E. coli</i> O157:H7	Ahmed et al. (2)	55	690	4.8
<i>S. typhimurium</i>	Mackey and Derrick (16)	55	440	3.3
<i>B. thermosphacta</i>	Baranyi et al. (5)	50	180	4.4

where  $N$  is the bacterial concentration (in CFU per milliliter) and  $D_{\text{ref}}$  is  $D$  at the reference temperature ( $T_{\text{ref}}$ ). Simulations were performed for *E. coli* O157:H7, *S. typhimurium*, and *B. thermosphacta*. The  $D$  and  $z$  values were obtained from literature (in part from work previously done at Reading) as shown in Table 1. The data for *E. coli* O157:H7 were obtained from inactivation experiments with ground beef, whereas the data for the other two organisms were obtained with liquid broth. All simulations were performed with the assumption that a cylinder with a diameter of 0.027 m and a length of 0.20 m was used. Simulation results are for a radial cross section taken from the middle of a cylinder. Sample simulations showed that owing to the high aspect ratio of the cylinder, the results were not significantly different along the length of most of the cylinder.

**Validation of heat transfer model.** To demonstrate that heat transfer within the agar sausage is accurately described by this model, agar cylinders were made (as described above, but uninoculated with bacteria) and halved so as to create two cylinders of half the original length. Each half-cylinder possessed a flat end (where the cut had been made) and a rounded end. A type K thermocouple (RS Components Ltd.), threaded through a Pasteur pipette such that it just protruded out of the thin end of the pipette, was inserted axially into the agar through the flat end of the cylinder. Only cylinders in which the radial distance of the thermocouple from the centers of the cylinders was 10% of the radius of the cylinders were used in heat transfer experiments. These agar cylindrical blocks were then immersed (vertically) into a water bath. The temperature change (accuracy,  $\pm 0.1^\circ\text{C}$ ) over time was recorded and compared to the theoretical temperature change.

## RESULTS AND DISCUSSION

**Validation of heat transfer model.** Heat conduction into the agar cylinder was modelled by using equations 1, 2, and 4. Experimental validation of this model can be seen in Fig. 2, which illustrates a typical temperature profile with  $r/R$  equal to 0.1 for an agar cylinder heated at  $67.3^\circ\text{C}$ . It can be seen that excellent agreement between theoretical and experimental results was obtained. The closeness of fit confirms (i) that there is little to be gained by way of increased accuracy in modelling by the use of an external heat transfer coefficient and (ii) that the other physical properties used in the model provide a good description of the heat transfer properties of the agar cylinder. There is still a need to identify the validity of the model and details of data in all circumstances.

**Numeric simulations of the thermal inactivation of *E. coli* O157:H7.** Numeric simulations were performed to determine the destruction of *E. coli* O157:H7 in a ground beef sausage (at an initial temperature of  $25^\circ\text{C}$ ) exposed to external heating for

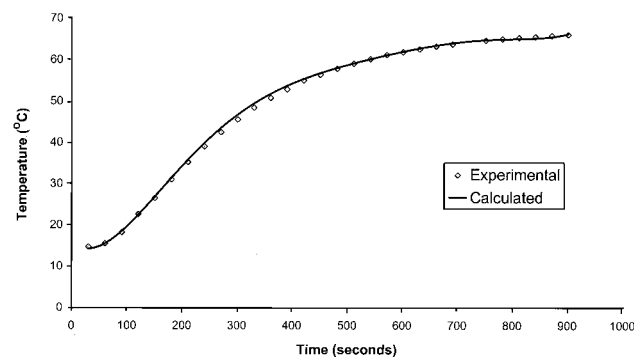


FIG. 2. Profile of the temperatures inside an agar cylinder, showing experimental results and the fit of the mathematical model to data.

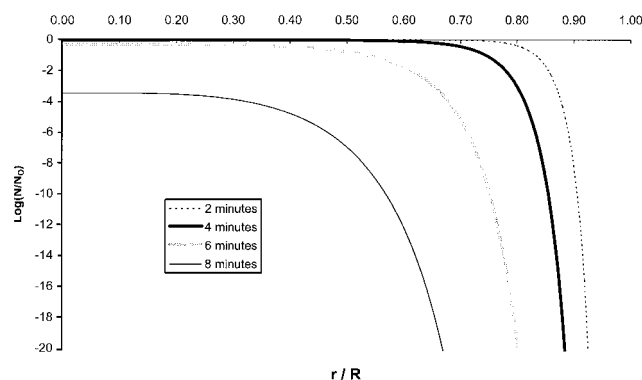


FIG. 3. Effect of exposure time on predicted *E. coli* O157:H7 inactivation along the radius of a ground beef sausage exposed to  $70^\circ\text{C}$ .

a specified time followed by cooling (at  $20^\circ\text{C}$ ) to a point where bacterial inactivation is no longer significant. The model parameters used in the simulations are listed in Table 1. Figure 3 shows profiles for predicted bacterial inactivation as a function of  $r/R$  within such a sausage exposed to a temperature of  $70^\circ\text{C}$  for different heating times. As heat penetrates into the sausage, bacterial count begins to rapidly drop off. Wherever  $\log(N/N_0)$  (where  $N_0$  is the initial bacterial concentration) was less than  $-2$  (2D inactivation) within the sausage, the temperature at some point in the process rose above  $61.0^\circ\text{C}$ , at which point *E. coli* O157:H7 has a  $D$  value of 38 s. At  $60.0^\circ\text{C}$  or less, the  $D$  value is greater than a minute, thus indicating the sharp sensitivity of bacterial inactivation rates to small changes in temperature. Figure 3 also shows that at early heating times, there was a steep gradient of inactivation as a function of distance from the center but that at later times, the effect of position on degree of inactivation was less (when the profiles for 2 and 8 min are compared).

Figure 4 shows the effect of different heating temperatures ( $65$ ,  $70$ , and  $75^\circ\text{C}$ ) on inactivation of *E. coli* O157:H7 as a function of exposure time in the center of a sausage. Clearly, it is important to pay attention to the effects of both time and temperature in thermal processing to identify the critical regions which lead to such a rapid drop in bacterial numbers. Figure 4 shows that the critical time interval over which viable numbers decrease rapidly is much narrower at  $75^\circ\text{C}$  than at  $65^\circ\text{C}$ . Thus, at  $65^\circ\text{C}$  numbers at the center began to decrease after 5 min, but a 7D inactivation was not achieved until 12

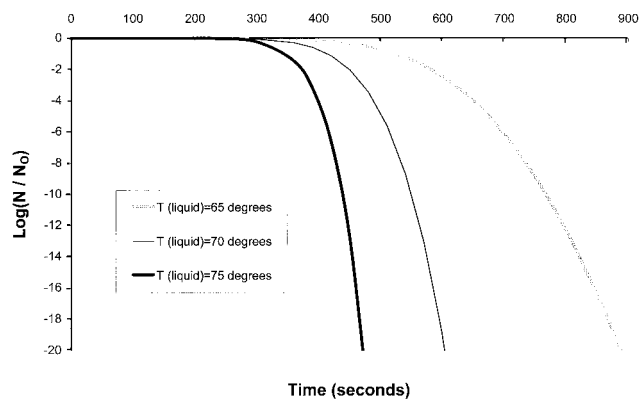


FIG. 4. Predicted *E. coli* O157:H7 inactivation in the center of a ground beef sausage at different heating temperatures.



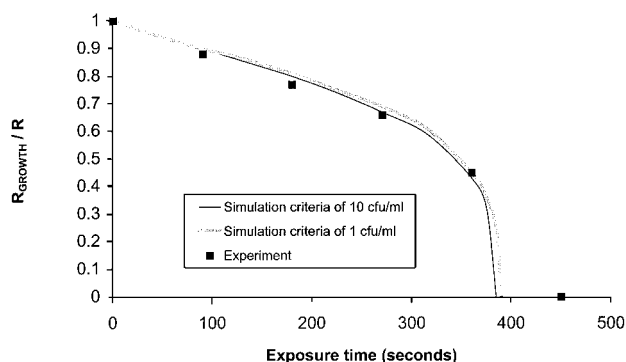


FIG. 5. Experimental validation of the inactivation of *S. typhimurium* in an agar cylinder (exposure temperature, 70°C).

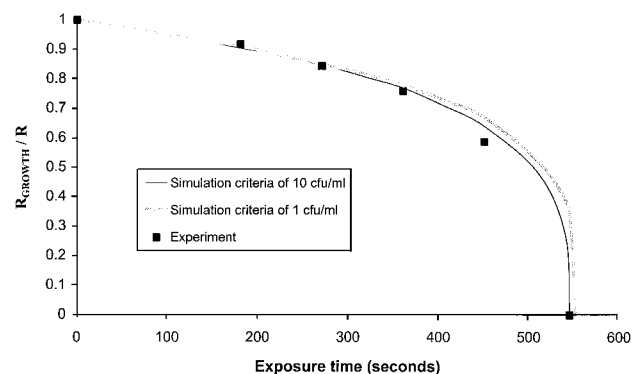


FIG. 6. Experimental validation of the inactivation of *B. thermosphacta* in an agar cylinder (exposure temperature, 60°C).

min, whereas at 75°C viable numbers began to decline at about the same time (5 min), but a 7D inactivation was reached only 2 min later.

It is also important to note the effect of the cooling period on bacterial inactivation. As the sausage is already loaded up with heat, heat continues to diffuse into the center of the sausage even as the outside is being cooled. Consider results after 9 min of heating at 70°C (Fig. 4). After the heating period, the center of the cylinder reaches a temperature of 61.7°C and a 1.9D inactivation is produced. After cooling, an inactivation of 8.7D is attained. A peak temperature of 63.2°C was reached during thermal processing of the sausage.

**Measurement of bacterial inactivation in agar cylinders.** Figure 1 shows examples of slices taken from TSA sausages inoculated with *S. typhimurium* which were externally heated in a water bath at 70°C. A distinction can be seen between darker regions, where surviving bacteria have regrown, and clearer regions on the outside, where thermal inactivation took place. The centers of the cylinders in Fig. 1 appear somewhat lighter than the surrounding regions. This was believed to be a consequence of oxygen depletion by bacteria closer to the perimeter of the slice. This effect creates an anaerobic environment in the center of the cylinder which restricts the extent of growth in this region. Fortunately, for the purposes of this study, it is necessary only to visualize the outer perimeter of bacterial growth.

The initial bacterial concentration within the agar sausage was known. However, in order to compare experimental data of the type in Fig. 1 with model predictions, one needs to have a defined bacterial concentration below which one would not expect to see a dark region on an agar slice. To this end, BHIA cylinders were made (as described in Materials and Methods) with diluted *B. thermosphacta* inoculum to produce bacterial concentrations of 10, 100, and 1,000 CFU/ml. In the instance of a concentration of 1,000 CFU/ml, dark slices were produced. With a concentration of 100 CFU/ml, sausage slices appeared cloudy, with a large number of colonies suspended in the agar. At 10 CFU/ml, slices were much clearer, with few colonies visible. The threshold for bacterial inactivation, as viewed under the image analysis system was thus assumed to be within the range 1 to 10 CFU/ml.

Experimental data from the growth zone radii of slices taken at differing thermal exposure times are shown in Fig. 5 and 6 for *S. typhimurium* and *B. thermosphacta*, respectively. The results of simulations of bacterial inactivation for which bacterial concentrations of 1 and 10 CFU/ml were used as threshold values for inactivation are also shown in these graphs. For

both bacteria, the simulations document inactivation of bacteria within the agar cylinder extremely well.

The mean deviation of experimental data from that predicted by theory is 2.9% if we assume a 10-CFU/ml criterion and 3.6% if we assume a 1-CFU/ml criterion. Finite-difference simulations use mesh spacings for  $r/R$  of 0.01. The experimental error involved in evaluating  $R_{\text{growth}}/R$  by image analysis was also believed to be approximately  $\pm 1\%$  after slices taken from the same cylindrical agar block were examined. Hence, differences between theoretical and experimental values for growth zone radii were not thought to be significant. Simulations also indicated little deviation between the results for 1- and 10-CFU/ml criteria, which is to be expected, as simulations shown in Fig. 3 indicate a very rapid drop in bacterial count after the temperature reached a critical point within the solid.

It is evident from the data that the integrated lethal effect of a dynamically varying temperature within the agar cylinder can be used to predict bacterial kill based on isothermal data. This lethal effect is believed to be a consequence of the relatively high rates of heating involved in this study. Under conditions of low heating rates (2°C/min or less), *S. typhimurium* has been shown to exhibit increased heat resistance as a consequence of heat shock-induced thermotolerance (17). Heat shock-induced thermotolerance may become significant when food is heated up very slowly, i.e., at rates an order of magnitude less than the heating rates used in this study.

Agar forms a macroreticular network of several sideways linked helices whose ends are joined together randomly to form pores. At agar concentrations of 0.7% (wt/vol) or greater, bacteria become immobilized (30). Given the closeness of agreement between experimental and theoretical results, our results suggest that immobilization within agar does not affect bacterial thermal resistance. The implication of this conclusion is that agar can be used as a model food to immobilize bacteria and experimentally validate the modelling of bacterial thermal destruction in process systems. A pertinent example of this would be heat transfer to a flowing food-liquid suspension (18).

Conventional food processing operations entail heating food until the center reaches the desired lethal temperature, at which point heating is continued for a period of time sufficient to ensure adequate microbial killing, followed by cooling. In some processing operations, for example, the tuna canning process (4), this procedure has been found to overprocess food nearer the outside of the container. A suitable knowledge of the heat transfer properties of food and the necessary ap-

proaches to process modelling can help ameliorate this problem, as it has already been demonstrated, with numeric simulations, that the cooling period can contribute considerably to the destruction of bacteria at the center of food. Furthermore, Fig. 5 and 6 exhibit a sharp decrease in  $R_{\text{growth}}$  with exposure time. The ability to predict this point is useful, as it will allow one to optimize thermal inactivation while minimizing the overprocessing of foodstuffs.

The modelling of heat transfer and the consequent microbial kill in real foods, however, is a major challenge because the physiochemical composition of food can affect both heat penetration and the thermal resistance of microbes. For example, Franz and von Holy (12) found that the fat contained in vacuum-packed sausages provided a significant protective effect for lactic acid bacteria when the sausages were exposed to heat. It has also been demonstrated that the thermal properties of foodstuffs can vary extensively when different samples are compared, which in turn has a pronounced effect on temperature profiles within foods (19). Nonetheless, Nicolai et al. (20) demonstrated that it was possible, using finite-element modelling, to model heat conduction in lasagna using steam heating. More comprehensive data on the thermal properties of food components and more systematic data on thermal resistance under differing environmental conditions (water activity, pH, fat content, etc.) will help provide a solid basis upon which to predict the safety of cooked or thermally processed foods.

The study presented in this paper modelled and experimentally validated thermal inactivation of the bacteria *S. typhimurium* and *B. thermosphacta* immobilized in an agar cylindrical block. While previous studies have pointed out the importance of finite-difference modelling for predicting bacterial inactivation spatially within a solid object exposed to conduction heating (26), this is the first study to visually demonstrate it with bacteria. The good agreement between model simulation and experimental data demonstrates well the appropriateness of the application of mathematical modelling in food microbiology.

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